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A CHEMICAL AND PHYSIOLOGICAL STUDY OF MOTTLING OF LEAVES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **277**

F. M. SCHERTZ

(WITH SIX FIGURES)

Introduction

During the year 1917 SAMPSON (**43**) used *Coleus Blumei* (var. Golden Bedder) for studying the chemistry and physiology of leaf fall. He noticed ·that when the leaves were ready to drop they had completely or almost completely lost their chlorophyll, and that in general they were inclined to lose their chlorophyll or to mottle. It was thought that this accentuated material might be excellent for the study of the factors involved in mottling of leaves in general.

Loss of chlorophyll from plant organs is a very general phenomenon. It is thought of as an orderly and natural thing in autumnal coloration, but is considered a diseased condition when it occurs during the growing season. In the latter case it is spoken of as mottling or chlorosis. It is not at all improbable that a study which throws new light on mottling will also illuminate autumnal coloration. Indeed, this work indicates that the two have many things in common.

BORESCH (**6**) found that algae growing for some time on nutrient solutions gradually changed from a dark green to gold or red brown. If nitrate solution was added to these cultures, the algae regained

their normal green color. Extracts made from the algae showed that chlorophyll decreased while carotin increased in the algae as they browned. ARTARI (2) grew algae in the dark and found that with an organic source of nitrogen they remained green, but with potassium nitrate as the source of nitrogen the algae lost their green color. With potassium nitrate as the source of nitrogen the colorless algae would regain a normal green when placed in the light.

SWART (48), working on spring and autumn leaves, found that just as the leaves were ready to fall they usually became yellow, and this was accompanied by a decrease in protein, nitrogen, phosphorus, and potassium. WILLSTÄTTER (53) noticed that in autumn, as the leaves yellowed, their chlorophyll content became less.

BRIGGS, LYMAN, JENSEN, and McLANE (7) have summarized the suggested causes of mottling in various plants. Excess of lime, magnesium, organic matter, or some essential element, deficiency in lime, iron, organic matter, or some essential element, low humus, high nitrogen, inorganic manures, frost, poor drainage, wind, sunlight, irregular supply of plant food and moisture, fungi or bacteria, nematodes, a filterable virus, and other causes are claimed by some to produce mottling. Chlorophyll may disappear owing to the absence of some essential constituent in the leaf, or to the presence of some deleterious substance. They thought that the soil was the cause of the mottling of citrus leaves and consequently analyzed it. It was found that mottling diminished as the humus ratio increased. Most of the trees which bore mottled leaves grew in soil which had a low nitrogen content. They believed that the mottling of the orange trees was definitely correlated with the low humus content of the soil, for mottling diminishes as the humus content increases. Alfalfa and bean straw were recommended for use in mulching the citrus trees.

JENSEN (25) analyzed green and mottled leaves, since it is known that organic matter attacks the soil minerals and sets free Ca, Fe, Mg, and PO_4, because these elements are closely connected with the formation of chlorophyll. It was found that badly mottled leaves from orange and lemon trees always contained a higher percentage of iron, calcium, magnesium, and phosphorus than the

healthy green leaves. Leaves in the medium stage of mottling sometimes contained more and sometimes less of these four elements. Midribs of the healthy leaves contained less of these elements than the mesophyll, while in badly mottled leaves the midribs contained more calcium and more phosphorus. The petioles contained less iron, calcium, and magnesium than either midrib or mesophyll in healthy and mottled leaves, while in badly mottled leaves the petioles contained more iron, calcium, and magnesium than either the midrib or mesophyll. Old leaves were found to contain more calcium and magnesium than new leaves. The yellow spots in the mottled leaves contained less calcium, magnesium, and phosphorus than the green parts of the same leaf. In working on the golden privet JENSEN found that the yellowed leaves contained more iron and 2.5 times as much phosphorus as the green ones. The increased amount of iron, calcium, magnesium, and phosphorus in the conducting tissues of the badly mottled leaves indicated that there was difficulty in the transfer of these materials to and fro.

McBETH (37) found that plots receiving large applications of commercial fertilizers generally bore trees with badly mottled leaves, while trees receiving no nitrogen or barnyard manure generally showed little mottling. In other groves extreme mottling was frequently associated with a high nitrogen content. Moisture and nitrogen content of the mottled leaves were found to be higher than in the normal green leaves. His work seemed to indicate that too much nitrogen caused the mottling of citrus trees.

Investigation

The mottling of *Coleus* leaves occurs in a regular manner, proceeding from the edge inward and toward the base of the leaf. The edge usually yellows first, while only in rare cases do yellow spots develop in the central part before the edges become yellow. The basal portion seems to retain its green color longer than the tip, and the veins or the region near the veins are the last to lose their green color. The leaves on plants in good soil often die at the tip about the time of mottling, while the leaves on plants in poor soil rarely show this characteristic. Usually the leaves from plants

in good soil only partially yellow before they drop, while the leaves on plants in poor soil always completely yellow before dropping. This might suggest that mottling and dropping involve different factors.

MEYER (38) points out that leaves of *Tropaeolum* passed through the following stages: dark green 25 days, green 6 days, bright green 12 days, yellow green-yellow 3 days, and then bright yellow. The young leaves at the top of the stem were dark green, while those at the bottom were yellow or wilting. The yellowing he believed to be due to the aging of the leaves. The change from bright green to yellow green was very rapid, and took place in much the same manner as that described for *Coleus*.

MORPHOLOGICAL EXAMINATION

In comparing microscopically the green with the mottling leaf, several striking differences were observed. In the green leaf the chloroplasts were large and blue-green, and one to three or more starch grains were clearly visible in the chloroplasts. The guard cells seemed to retain their coloring matter longer than the adjoining cells. In the mottled leaf the chloroplasts were yellowish, fewer in number, much smaller, without a green tint, and without starch grains. The chloroplasts were clustered about the apparently normal nuclei or distributed throughout other parts of the cells. Also the general appearance of bacteria being active here was observed, and will be discussed later. The chloroplasts in the palisade cells of the normal green leaves were 2–5 μ in diameter, while those in the mottled leaves were 1 μ or less in diameter. The chloroplasts in the guard cells of the green leaves, as well as those in the mottled leaves, were about 1 μ in diameter. SWART observed that in the aging of leaves the chloroplasts broke down and the starch disappeared, but the nuclei and the plasma layers remained. He was not certain whether the chlorophyll escaped from the cell or not. The chloroplasts of the deep green leaves of *Tropaeolum majus*, as noted by MEYER, were larger than those of the pale green leaves. Since he made some very accurate determinations of the sizes of the chloroplasts and correlated the size with the color of the leaves, it will not be out of place here to quote him rather fully.

MEYER found that the protein of the palisade cells is located chiefly in the chloroplasts, which he looks upon as the birthplace of the proteins, hence one can see why the color of the leaves and the protein content are so intimately related. He observed also

TABLE I

COMPARISON OF CHLOROPLASTS OF PALISADE CELLS IN *Tropaeolum*

Color of leaves	Deep dark green	Deep green	Green	Bright green	Yellow
Relation of diameters....	126	100	86	72	52
Relation of volumes.....	200	100	64	38	14

that as the leaves yellowed there was little change in the size of the nucleus, the nucleolus, or in the protein content of the cytoplasm. He inferred that the formation of chlorophyll in the chloroplasts follows the development of protein in the leaves.

CULTURES

The plants were grown in the purest fine quartz sand, in new 4-inch flower pots. Experiments were conducted in which the sand was watered with nutrient solutions bearing all of the necessary elements for plant nutrition, or lacking either Ca, Mg, P, Fe, or N. Twenty-six plants were used in each of the six sets, making 156 in all. The set of plants receiving the complete nutrient was watered with Pfeffer's solution as given by DUGGAR (14). When iron, calcium, magnesium, phosphorus, or nitrogen was omitted from the complete solution, the salts suggested by DUGGAR were substituted. In the solution lacking iron, $Ca(NO_3)_2$, KNO_3, $MgSO_4$, KCl, and KH_2PO_4 were used; in that lacking magnesium, KNO_3, $Ca(NO_3)_2$, Na_2SO_4, $FeCl_3$, KH_2PO_4, and KCl; in that lacking phosphate, $Ca(NO_3)_2$, KNO_3, $MgSO_4$, KCl, and $FeCl_3$; in that lacking nitrate, $CaCl_2$, KCl, $MgSO_4$, KH_2PO_4, KCl, and $FeCl_3$; in that lacking calcium, $NaNO_3$, KNO_3, $MgSO_4$, KH_2PO_4, KCl, and $FeCl_3$. All of these were made up according to DUGGAR. Cuttings were made of the plants, which were then rooted in sand for two weeks. The freshly potted plants were watered first with nutrient solutions and then every morning with distilled water. About every week another application of the nutrients was made. The plants were

grown from May 1 to August 1. The effect of lack of nitrogen was evidenced in four or five days by noticeable yellowing.

Fig. 1 shows the condition of the various cultures on August 1. The plants with complete nutrient solution, and those without calcium, magnesium, or iron, grew about equally well. There was evidently enough of each of these elements already in the cuttings to care for considerable additional growth. All these plants had a good green color, indicating a plentiful chlorophyll supply. The effect of the absence of PO_4 or NO_3 was especially striking. A considerable nitrogen and phosphate supply evidently was necessary
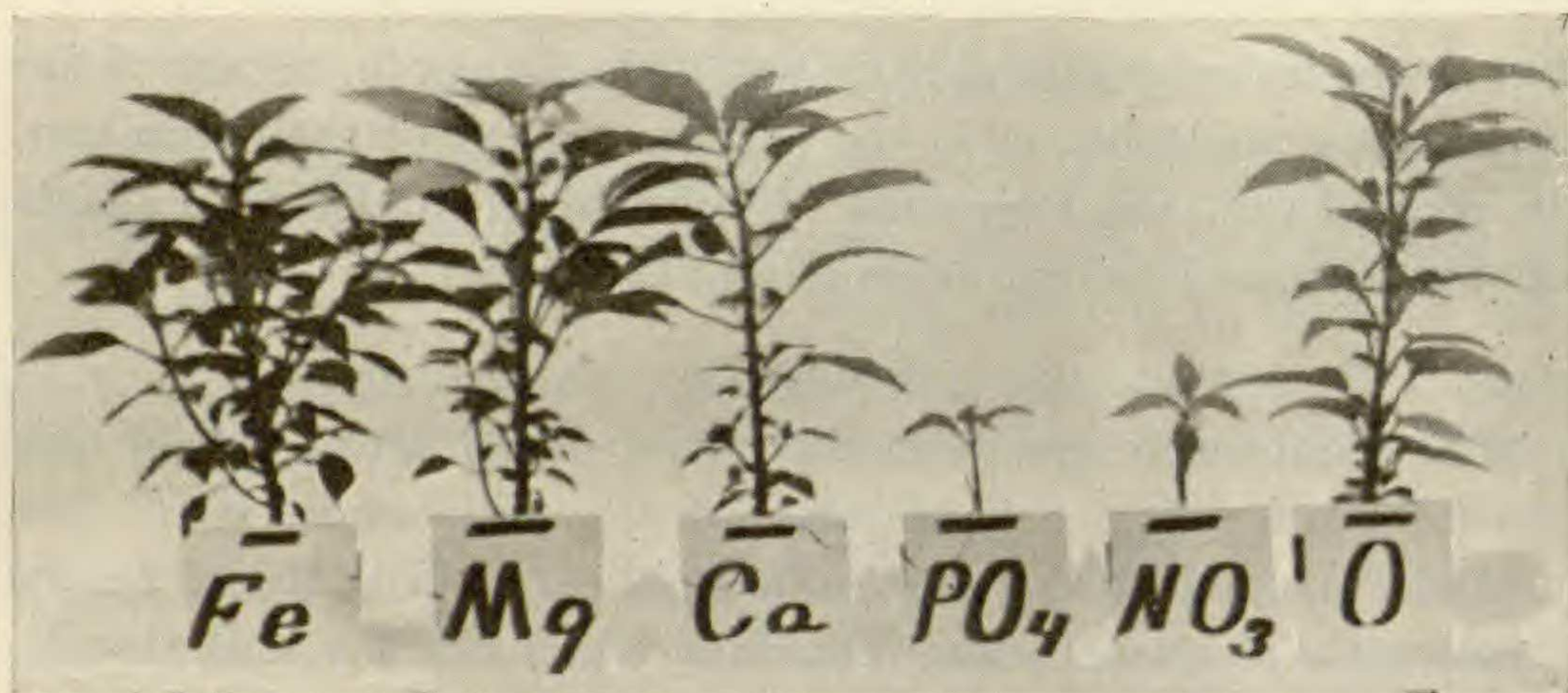

FIG. 1.—*Coleus* plants grown in various nutrient solutions: −Fe, iron lacking but all other essential elements present; −Mg, magnesium lacking, etc.; −O, all essential elements present; notice dwarfed condition of plants lacking phosphate or nitrate.

to cause any increment in the growth of the plants. The plants grown without PO_4 were very small, but the leaves were a deep green, even greener than any of the plants in the best soil. While addition of phosphorus was needed for any considerable growth of the plant, it was not needed for the maintenance of the chlorophyll. The deep green in the phosphorus-lacking plants probably was due to the high nitrate supply in proportion to the size of the plant, for here, as in all of this work, nitrate supply or its deficiency seemed to determine the development or the disappearance of chlorophyll. The plants grown in solutions lacking one of the elements Ca, Mg, or Fe, and those in complete nutrient solutions

all showed branches developing in axils of the leaves. The branching was especially prominent in the plants watered with nutrient solution lacking iron. No branches developed on the plants watered with nutrient solutions lacking PO_4 or NO_3.

On August 1 (3 months after planting) the number of pairs of leaves still attached was counted and compared with the number of pairs which had fallen. The plants were similar when the experiment was begun. Each group contained 26 plants and the average of these was taken.

Table II shows that when phosphate or nitrate was lacking a greater percentage of the leaves fell compared with any other element. This may partially be accounted for because plants lacking NO_3 or PO_4 had a smaller percentage of new leaves, and

TABLE II

EFFECT OF NUTRIENT ON LEAF FALL

Leaves	Element lacking					
	Fe	Mg	Ca	PO₄	NO₃	None
Average number of pairs dropped per plant......	7	7	6	7	5	6
Average number of pairs still attached..........	9	9	9	3	4	9
Percentage dropped.......	45	45	40	70	56	40

consequently a smaller percentage of leaf fall. This did not account for the fact that when phosphate was lacking 14 per cent more of the leaves fell than when nitrate was lacking. The plants lacking Fe and those lacking Mg during the course of the experiment grew 16 pairs of leaves; those lacking Ca and those on complete nutrient solution each grew 15 pairs of leaves; those lacking PO_4 grew 10 pairs of leaves; and those lacking NO_3 grew only 9 pairs.

To 8 of the plants (2.5 months old) which were grown in sand cultures with NO_3 or PO_4 lacking, one watering was made with a solution which contained the lacking element. The effect is shown in fig. 2. Two weeks after the watering the height of the treated plants was about twice that of those which had no nitrate or no phosphate added, and the area of the new leaves put forth was from three to four times the area of the old leaves below them.

The plants to which nitrate was added showed noticeable greening in 4 or 5 days, and somewhat later became a normal green. Not only did the new leaves put forth become green, but even the light yellow leaves which were on at the time the nitrate was added became green.

Another set of experiments was carried out in which the elements Mg, N, P, Ca, and Fe were added to plants growing in pots in ordinary potting soil (figs. 3, 4). The solutions used were 2 per cent $FeCl_3$, 1 per cent $MgCl_2$, 6 per cent $CaCl_2$, 2 per cent KH_2PO_4, and 8 per cent $NaNO_3$. Eight plants were used for each treatment and 8 for controls, making 48 in all. In each case 0.25 cc. of the salt solution, diluted to 6.25 cc., was applied to the soil in the pots three times a week. In addition to this the plants were watered daily with tap water. The plants to which iron was added were given two or three drops of the iron solution each week. Some of the plants before being placed under treatment had already begun to mottle, but those to which nitrate was added rapidly regained their normal green. All of the plants to which nitrate was added retained their normal green and held their leaves better than the other cultures; also they branched and were sturdier than the rest. The plants shown in fig. 3 were grown 4 months in 2-inch pots, while those shown in fig. 4 were grown 4 months in 3-inch pots. Plants which were used as controls did not seem to do as well as the others, while the plants which received phosphate lost a large percentage of their leaves. The plants which received magnesium, calcium, or iron grew about equally well. The data given in table II show that lack of phosphate seemed to cause leaf fall, while here its addition caused the same effect. The lack of phosphates caused the leaf to fall, while the addition of phosphates alone to the soil, the nutrients not being present in a balanced ratio, produced the same effect.

DICKSON'S (12) work on oats is of interest in this connection. He found that oat seedlings grown in solutions deficient in phosphorus or nitrogen produced but one slender shoot. Plants grown in solutions deficient in Ca or Mg stooled heavily before those grown in complete nutrient solutions, and later the plants grown in solutions deficient in magnesium showed marked striping

FIG. 2.—Plants 3 months old: one labeled $-NO_3$ grown full time with NO_3 lacking in nutrient solution; one labeled $+NO_3$ grown with NO_3 lacking in nutrient solution for 2.5 months and then one dose of NO_3 added, after which plants grown for 2 weeks more; plants labeled $-PO_4$ and $+PO_4$ similarly treated; here PO_4 was substance lacking or added.

FIG. 3.—Plants 4 months old, during which time solutions containing Mg, N, P, Ca, Fe, or nothing were added to soil in respective pots; effect of addition of N shown by greater development of plant, as well as healthier color of leaves.
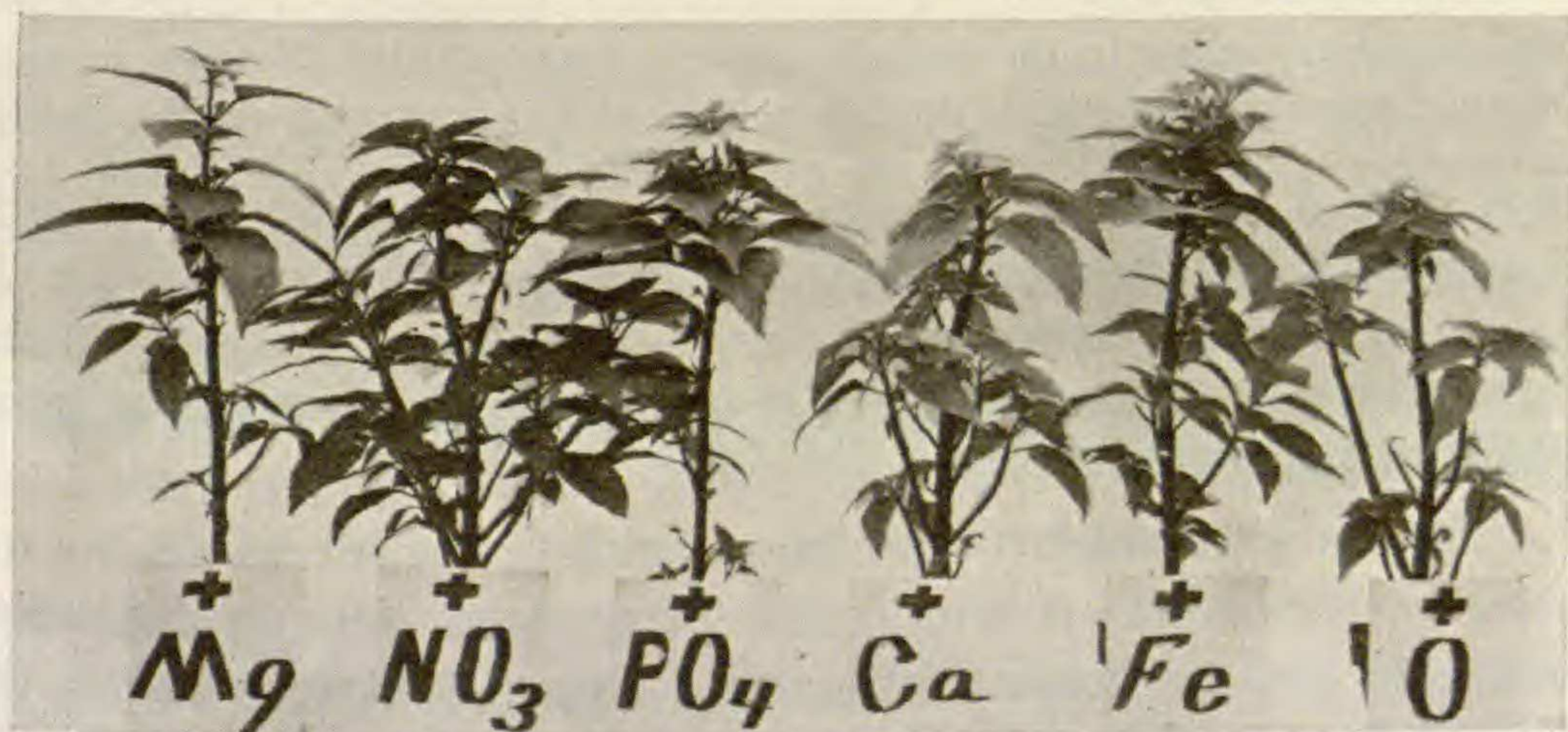

FIG. 4.—Plants similar to those in fig. 3, but grown in pots 1 inch larger

as the chlorophyll disappeared between the veins. Leaves and stems of plants grown in culture solutions deficient in phosphorus were purplish green. This color is apparently due to the presence of a purplish colloidal substance (perhaps a result of the decomposition of the chlorophyll) intermixed with the chlorophyll. Oat plants grown in solutions weak in nitrogen produced narrow purplish green leaves. Microscopical examination showed the chloroplasts more or less disorganized. A deficiency in phosphorus or in nitrogen produced a markedly unfavorable effect by causing a great decrease in the vegetative growth.

JOST (27) asserts that two *Helianthus* plants growing to maturity in three months used 1.4 gm. of KNO_3. These experiments with *Coleus* plants showed that one *Coleus* plant growing in soil in a 3-inch pot used the equivalent of 1 gm. of KNO_3, in addition to the nitrate which was in the soil, and then did not reach maturity. This shows that *Coleus*, being a plant much smaller than the sunflower, seems to use large quantities of nitrate. During the course of the experiment, if the nitrogen supply was discontinued at any time, the plants began to mottle at the margin of the leaves, but the leaves greened when the supply was again added. This shows that the plant was using the nitrate which was added to the soil in the pot. BURD (9) computed from the crop the amount of nitrate one barley plant used, and found that about 1.1 gm., calculated as potassium nitrate, was sufficient to bring the plant to maturity. PALLADIN (40) states as follows:

Carbohydrates are essential to the formation of chlorophyll. Plants fall into two groups according to the carbohydrate content of their etiolated leaves. In one group (for example, barley) the etiolated leaves contain much soluble carbohydrate material, while in the other group (as beans and lupines) the etiolated leaves contain very little carbohydrate. If etiolated leaves of these plants are removed and floated upon water in the light, those of barley become green, while almost all the bean leaves and all those of lupine remain yellow. If the latter are floated, not upon water but upon a saccharose or glucose solution, then they also become green.

The writer conducted experiments with *Coleus* leaves similar to these of PALLADIN. Young *Coleus* plants were kept in darkness until the leaves became etiolated. These etiolated leaves of *Coleus* when floated on distilled water in the light remained yellow,

those on KNO_3 solution died, while those on a 5 per cent sugar solution greened slightly. On the other hand, *Coleus* leaves which had mottled in the light did not green again when floated on any of these solutions. In the case of etiolated *Coleus* leaves it apparently is true that they do not form chlorophyll because of the lack of carbohydrates. The case of the mottled leaves cannot be explained on this basis. The catalase experiments (see later) show that the vigor of the mottled leaves is greatly reduced, and this would account for the inability of the mottled leaves to green again. In the case of mottled leaves carbohydrates are evidently not the limiting factor, for tests for carbohydrates showed that plenty of starch was present in the leaves.

Microchemical analysis

The differences between the normal green and the mottled leaf were determined by microchemical methods. The tests used were those given by MOLISCH and TUNMANN in their texts on microchemistry, and generally several tests were applied in order to determine the presence or absence of a substance.

Tests for starch were made upon the two types of leaves. Green and mottled leaves gathered before 8 o'clock (sun time) showed a wide variation. The guard cells of the mottled leaves were especially full of starch, while some starch was found in the other parts of the leaves; the whole leaf had a bright blue color after testing with iodine. Much starch was found in all parts of the green leaf, and it was colored deep violet to black by the iodine used. In the evening, after a bright sunny day, leaves were collected and the amount of starch again determined. The guard cells of the mottled leaves were well filled with starch, and the other cells had much more starch than they had in the morning. The chloroplasts which were present appeared to be active in forming starch, even though no chlorophyll seemed to be present. Since this investigation the writer has had occasion to make some very accurate tests for small amounts of chlorophyll, and it is doubtless true that if solutions of the pigments of mottled leaves of *Coleus* had been subjected to spectrophotometric tests, chlorophyll would have been discovered, at least in small amounts. All cells

of the green leaf were completely filled with starch, which was present in larger grains than it was in the mottled leaves. The masses of starch here appeared to be about five times the diameter of the masses in the mottled leaves. At noon, a healthy plant which had the lower leaves mottled was placed in the dark. Immediately one-third of the tip end of one of the green leaves was cut off, then in 6 hours another one-third was cut off, and at the end of 18 hours the remainder of the leaf was removed. The same was done with the mottled leaf.

Table III shows that the translocation power of the leaf was still active, and proves that diastase was not inhibited by oxidizing enzymes, as was believed by WOODS (18) in the case of mosaic leaf of tobacco.

TABLE III

TRANSLOCATION OF STARCH

Leaf	Placed in darkness	After 6 hours	After 18 hours
Green	Much	Medium	Little
Mottled	Fair amount	Less starch	Minute traces

Tests for iron in the chloroplasts were made with potassium ferrocyanide. Both the green and the mottled leaves had iron in their chloroplasts. The chloroplasts which were present in the mottled leaf were colored about as deeply as those in the green leaf. From the blue tint which was produced in the leaves, the green ones appeared to have more iron than the mottled ones. From the macrochemical results which follow, it is evident that some of the iron in the leaves is "masked."

In comparing the amount of ammonium magnesium phosphate crystals which were formed in the two leaves on the addition of sodium ammonium phosphate, there appeared to be slightly more crystals formed in the cells of the mottled leaf blade. In both green and mottled leaves less magnesium was found in the upper part of the petiole than in the part of the petiole nearest the stem. The petioles of young green leaves had about the same amount of magnesium as the petioles of strongly mottled leaves. In the petioles about one crystal of ammonium magnesium phosphate

per cell was formed in the cortex and in the pith. In the plant with mottled leaves 2 or 3 crystals were formed in each pith cell of the stem. Magnesium was also present in the xylem, phloem, cortex, and epidermal cells.

In making comparative tests of the leaves for calcium, apparently a few more calcium sulphate crystals were formed in the cells of the mottled than the green leaves. In the green leaves much calcium was found in the epidermis of the petioles, some in the xylem and phloem regions, and little in the parenchyma. The same was true of the mottled leaves. In the stems many calcium sulphate crystals were formed in the pith cells on the addition of H_2SO_4. If there was any difference, more crystals were formed in the stems of the mottled plants. Calcium sulphate crystals were also formed in the xylem, phloem, cambium, and cortical regions. The fact that the mottled leaves and stems were always older than the green leaves and stems of the same plant would account for more crystals being formed in their cells.

In testing for phosphates by the addition of ammonium and magnesium chloride, only a very few crystals of ammonium magnesium phosphate were formed in the green leaves or in the mottled ones. Evidently the phosphorus must have been in some organic form in which it is not readily reactive with the reagents used, hence no conclusion can be drawn from this test regarding the metabolic disturbances which may be produced by it.

The test for nitrates gave the most interesting result. All of the green leaves gave tests which showed that an abundance of nitrate was present, while no positive results were obtained from the completely mottled leaves. It is of value to compare the progress of the mottling of the leaf with the absence of nitrates. The first signs of mottling usually appeared at or near the lobes of the leaf, and it was here that the test for nitrates was first negative. At this stage the greatest amount of nitrates was found in the conducting tissues of the leaf. Also in the deepest green leaves the conducting tissues contained the most nitrate. As the green disappeared from the tip of the leaf, more and more nitrates were found only in the veins close to the base. At this stage only a little greenish tint remained in the leaves. Usually as long as the

veins still showed a greenish tint some nitrates were found to be present. The last traces of nitrate in the leaf were found only in the petioles. In a moderately yellowed plant whose leaves were very slightly green, nitrates were found only in the pith region at the base of the stem; hence nitrates began to disappear at the very tip of the leaves and were last found only at the base of the stem. The nitrates disappeared last in the storage regions.

Among other workers in microchemistry, SWART (48) found that in yellow leaves in autumn the amount of phosphorus, nitrogen, and potassium decreased shortly before the leaves fell. Comparing this with the mosaic disease of tobacco, FREIBERG (18) reports that more proteins were present in the lighter areas of the leaves than in the darker. Nitrates were present in about the same quantities in healthy and diseased areas. Ammonium salts, iron, calcium, magnesium, potassium, phosphorus, and sulphur were also present in the same quantities in the chlorotic and in the dark green areas. By employing Folin's micro-Kjeldahl method less nitrogen was found in the dark areas than in the lighter diseased areas. Diseased areas of the tobacco leaf gave a more pronounced reaction with Millon's reagent, the xanthoproteic reaction, and the biuret test than did the healthy areas. More carbohydrates were always present in the dark green or healthy areas.

Macrochemical analysis

Since the whole leaf of the plant mottled completely, it was easy to compare the green with the mottled leaves by an analysis of the leaves, including the petioles, for the presence or absence of the substances which were suspected of causing the disturbance. In making the following analyses, controls were always run on a known sample, and in many cases several methods of analysis were tried and the one which gave the best theoretical results was used.

In estimating the amount of iron present in the leaves, the method described by MARRIOTT and WOLF (34) was used. The blade, petioles, and region of the abscission layer were analyzed separately. A piece 3–4 mm. long was used for the analysis of iron in the abscission layer, and the petiole was cut off at the base

of the leaf. The leaves were taken from the same plants, which were grown in ordinary potting soil, dried to constant weight at 100° C., and then the dry material ashed at a low red heat. The iron was calculated as free iron.

The analysis for iron showed that the amount of iron increased in the abscission layer, in the petioles, and in the leaf blades in

TABLE IV

Comparison of amounts of iron in green and mottled leaves

Material	Wet weight	Dry weight	Percentage dry weight	Grams of iron	Grams of iron per gram of dry weight	Grams of iron per 100 grams of dry weight (average)
			Mottled leaves			
Abscission layer....	0.3450	0.0200	5.79	0.000,016	0.000,80	
	0.2585	0.0146	5.65	0.000,014	0.000,98	0.108
	0.2660	0.0166	6.24	0.000,024	0.001,47	
Petioles...........	2.0320	0.0865	4.25	0.000,042	0.000,49	
	1.5550	0.0582	3.74	0.000,036	0.000,62	0.042
	1.4080	0.0588	4.17	0.000,009	0.000,15	
Leaf blade.........	8.5660	0.4930	5.75	0.000,81	0.001,64	
	5.4694	0.3008	5.50	0.000,37	0.001,21	0.130
	6.4706	0.4520	6.98	0.000,48	0.001,06	
			Green leaves			
Abscission layer....	0.6450	0.0300	4.65	0.000,02	0.000,666	
	0.4224	0.0206	4.87	0.000,02	0.000,970	0.079
	0.3980	0.0192	4.82	0.000,02	0.000,729	
Petioles...........	4.7160	0.1690	3.58	0.000,045	0.000,266	
	2.4650	0.0992	4.02	0.000,038	0.000,383	0.030
	1.5780	0.0988	6.26	0.000,024	0.000,239	
Leaf blade.........	17.4220	1.2100	6.94	0.000,47	0.000,388	
	8.1016	0.5575	6.88	0.000,39	0.000,699	0.073
	10.3500	0.8080	7.80	0.000,90	0.001,110	

the mottled leaves. This result disagreed with the microchemical report, perhaps owing to the fact that the iron was bound in some way and was released only by ashing the leaf. It showed that about 1.55 per cent of the ash of the green leaves was Fe_2O_3. According to Palladin (40), beech leaves have 2.30 per cent of Fe_2O_3 in the ash, while Jost (27) states that tobacco leaves have 1.95 per cent. According to Palladin 0.09 per cent of the dry weight of pea

leaves and 0.11 per cent of the dry weight of bean leaves was Fe_2O_3, while *Coleus* leaves had 0.23 per cent as Fe_2O_3. The Fe_2O_3 in beech leaves increased from 0.8 per cent of the ash in May to 1.3 per cent of the ash in October. One concludes that the mottling of the *Coleus* leaves was not due to a deficiency in iron, for at all times the leaves had enough iron, when compared with

A   B   B   A

FIGS. 5, 6.—Fig. 5, plants from which leaves were taken for analysis: leaves missing on upper part of plant at left taken for analysis; lower leaves mottled and fallen off; lower pair of leaves still on each plant partially mottled; plant at left type *A*, plant at right type *B*; fig. 6, another group of plants from which leaves were taken for analysis: plant at right type *A*, plant at left type *B*; lower pair of leaves still on each plant partially mottled; plants grown close together to produce larger leaves, accounting for leaflessness of stems.

other plants, to carry on their metabolic activities in a normal manner. This fully agrees with the conclusions drawn from the cultures.

Two types of plants were used for the analyses which follow. Plants of type *A* were grown in 3- and 4-inch pots, until ready for repotting, and then put into 6- and 8-inch pots, using ordinary

potting soil for repotting. Plants of type B were taken from the same group as A, and were repotted in pots of the same size as A, but sand was used instead of soil. In this way the plants were grown under exactly the same conditions of light and moisture as were the plants of type A, but the amount of soil nutrient was considerably reduced. The larger plant in each figure (figs. 5, 6) is of type A. The two plants shown in fig. 6 were grown in 6-inch pots, while those in fig. 5 were grown in 8-inch pots. All the leaves designated "green" were picked from plants of type A, and the "mottled" leaves were picked from both sets of plants and were analyzed separately. The mottled leaves from plants of type A as a rule were only partially mottled, while those from type B were always completely mottled. The green leaves were picked from the plant 3 or 4 nodes above the yellowing leaves,

TABLE V

MAGNESIUM

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Per 100 gm. dry weight
Green....................	0.0922	0.0980	0.0952	0.0951	1.50
Mottled A..............	0.0883	0.0892	0.0935	0.0903	1.66
Mottled B..............	0.0938	0.0740	0.0840	0.0839	1.35

and the mottled leaves from types A and B were picked just about the time they were ready to fall. The leaves were always picked before 8:00 A.M. in order that the results might better be compared.

The amounts of magnesium and calcium were determined according to the methods given in Bulletin 107 of the Bureau of Chemistry (8). In each case 40 gm. of green material was used for the determinations, and the results are given in grams of the free element per 100 gm. of wet material. The leaves were dried at 100° C. before being analyzed, and the dry weights found were used in calculating the grams of calcium and magnesium per 100 gm. of dry weight.

The magnesium content of mottled *Coleus* leaves differed little from that of green ones, and the magnesium content of both was somewhat higher than that reported for other leaves. As calculated from table V, the ash of green *Coleus* leaves was 17.5

per cent MgO, while according to PALLADIN (40) that of beech leaves was 7.20 per cent MgO, and according to JOST that of tobacco leaves was 7.36 per cent MgO. PALLADIN states that 1.02 per cent of the dry weight of pea leaves was MgO, while 0.66 per cent of bean leaves was MgO. The writer found that 2.48 per cent of the dry weight of green leaves was MgO. In agreement with the results from cultures, the analytical data also indicated that the magnesium was in excess of the needs of the plant, and that decomposition of chlorophyll in these leaves was not due to a shortage in magnesium.

The amount of calcium present in mottled leaves of *Coleus* was slightly greater than that in green, and the calcium content of both was less than that found in tobacco and beech leaves, while it was more than that found in pea and bean leaves. As

TABLE VI

CALCIUM

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Per 100 gm. dry weight
Green..................	0.206	0.222	0.284	0.237	3.31
Mottled *A*.............	0.215	0.255	0.283	0.251	3.93
Mottled *B*.............	0.169	0.225	0.240	0.211	3.03

calculated from table VI, the ash of green *Coleus* leaves was 33.2 per cent CaO, while according to JOST that of tobacco leaves was 36 per cent CaO, and according to PALLADIN (40) that of beech leaves was 44.3 per cent CaO. PALLADIN found that 3.21 per cent of the dry weight of pea leaves was CaO, and of bean leaves 1.33 per cent was CaO. The increase of calcium (based on dry weight) from 3.31 per cent in the green *Coleus* leaves to 3.93 per cent in the mottled is easily accounted for by the fact that the older leaves have different ash constituents (44) from the young leaves. In beech leaves (40) the MgO content (based on dry weight) increased from 4.3 per cent in May to 5.6 per cent in July, and then decreased to 4.1 per cent in October. These data and the culture experiments showed that the amount of calcium present at all times was sufficient to care for the physiological needs of the plant.

Phosphates were determined by the NEUMANN-PEMBERTON (35) method and the result is given as free phosphorus. In each analysis 20 gm. of fresh leaves was used, and before being analyzed was dried at 100° C. in order to determine the dry weight.

The amount of phosphorus present in mottled *Coleus* leaves was considerably less than in green ones, and the phosphorus content of the green leaves was about the same as that of other leaves (tobacco and beech), but the phosphorus content of pea and bean leaves was higher than that of green *Coleus* leaves. As calculated from table VII, the ash of green *Coleus* leaves was 6.41 per cent P_2O_5, while according to PALLADIN (40) the ash of beech leaves was 7.80 per cent P_2O_5, and according to JOST the ash of tobacco leaves was 4.66 per cent P_2O_5. Of the dry weight of

TABLE VII
PHOSPHORUS

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of phosphorus per 100 gm. dry weight
Green.................	0.031	0.032	0.019	0.027	0.398
Mottled *A*..............	0.011	0.011	0.010	0.011	0.181
Mottled *B*.............	0.010	0.010	0.011	0.011	0.176

the green *Coleus* leaves, 0.91 per cent was P_2O_5, while according to PALLADIN pea leaves had 1.67 and bean leaves 2.19 per cent P_2O_5. With mottling the total percentage of phosphorus in the leaf of *Coleus* calculated as P_2O_5 (on ash basis) had decreased from 6.41 to 2.88 per cent, or somewhat over 50 per cent. In the case of beech leaves, PALLADIN found that in May the P_2O_5 content was 32.4 per cent of the ash, while in October it was only 5.1 per cent. The percentage decrease of P_2O_5 in *Coleus* leaves was not due to an accumulation of ash, but apparently to the decrease in the P_2O_5 before the leaf fell, for when one calculated the amount of P_2O_5 present in the green and in the mottled leaves, there was always a reduction of 50 per cent or more, whether the calculation was based on dry weight, wet weight, or ash. This reduction in the phosphate content of *Coleus* leaves was similar to that found by SWART (48) for other leaves, and in all probability the changes

which took place in *Coleus* are of a similar nature to those accompanying the yellowing of leaves in the autumn. Since the green and mottled leaves of *Coleus* were about the same size, and since there was only two or three weeks' difference at the most in the ages of the leaves of *Coleus*, it is hardly possible that the differences could be accounted for in any way except that the P_2O_5 content actually decreased, and this would mean a transfer of materials from the leaf to the stem. If the amount of phosphorus were figured per leaf, then, since the leaves were about the same size, there would be nearly a 50 per cent reduction in the amount of phosphorus. Such a conclusion is in harmony with results from the cultures, for they showed that phosphate was necessary for growth of the plant, and when phosphates were deficient a larger percentage of the leaves fell. This conclusion is not out of

TABLE VIII

PHOSPHORIC ACID CONTENT OF LEAVES OF *Acer Negundo*

Phosphoric acid	May 7	June 6	July 5	August 2	September 3	September 25
Percentage dry weight..	1.500	0.801	0.705	0.580	0.586	0.333
Grams per 200 leaves...	0.256	0.200	0.210	0.134	0.147	0.099
P_2O_5 as percentage of ash	20.8	10.7	8.8	6.3	6.2	2.9

harmony with the work of SCHULZE and SCHÜTZ (44). In working on *Acer Negundo* these investigators showed that the phosphorus content decreased gradually and quite definitely from May to September, whether the phosphorus content was calculated as percentage of dry weight, grams per 200 leaves, or calculated from the ash. Only the results of their work on leaves collected in the morning will be given here, as they are most directly comparable with those of the writer, yet it is worthy of note that nearly always the phosphorus content of the leaves in the evening was greater than in the morning. This seems to indicate storage and synthesis during the day and a loss of phosphorus compounds during the night. The phosphoric acid content of the leaves is summarized in table VIII.

This work shows that the amount of phosphoric acid in the leaves of *Acer Negundo* decreases as the season advances. From these data one cannot agree with WEHMER or RIESMÜLLER (as

cited by PALLADIN 41), who believe that the absolute amounts of phosphoric acid do not diminish as the season progresses. The writer must agree with SCHULZE and SCHÜTZ, who find that the phosphoric acid content decreases as the leaves age or as autumn approaches.

Further evidence for the correctness of these views is presented by TUCKER and TOLLENS (49), who have shown that phosphorus decreased markedly in the leaves of the plane tree on or about October 8. The amount of phosphorus in the leaves at the end of the growing season was less than 50 per cent of that found in the leaves during the earlier part of the year. They believe that the three plant nutrients (nitrogen, phosphorus, and potash)

TABLE IX

PROTEIN NITROGEN

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of nitrogen per 100 gm. dry weight
Green..................	0.189	0.243	0.207	0.213	3.35
Mottled A.............	0.076	0.080	0.087	0.081	1.39
Mottled B.............	0.073	0.064	0.079	0.072	1.14

passed from the leaves into the stems as the leaves aged, and were not washed out by rains.

Protein nitrogen was determined by the Gunning-Arnold modification of the Kjeldahl process. In each case 10 gm. samples of the fresh leaves were used, and the results as given in table IX are grams of nitrogen per 100 gm. of fresh leaves.

The green leaves contained 1.33 per cent protein (N×6.25), while the mottled leaves contained 0.51 per cent, and those on poor soil B 0.45 per cent, when calculated on the wet weight of the leaves. Based on the weight of the dry leaves, the green ones contained 20.9 per cent, the mottled A contained 8.7, and B 7.13 per cent protein. PALLADIN found considerable variation in the percentage of total nitrogen and protein nitrogen between etiolated and green leaves of various plants, but the difference was not always in the same direction. OTTO and KOOPER, and LeCLERC DU SABLON (19) found that leaves decrease in their protein content

from spring to autumn. It is evident that the protein content of green and mottled *Coleus* leaves does not parallel that of the green and etiolated leaves of PALLADIN. If it resembles the protein change with progress of the growing season, it is at least much more rapid. It is probably quite similar to the rapid changes just preceding leaf fall in autumn.

The work of SCHULZE and SCHÜTZ (44) may again be relied upon to show the normal changes which take place in *Acer Negundo*.

TABLE X

NITROGEN PRESENT IN *Acer Negundo* LEAVES AT VARIOUS TIMES OF YEAR

Protein N	May 7	June 6	July 5	August 2	September 3	September 25
In 200 leaves	0.734	0.973	1.211	0.864	0.791	0.628
Percentage in dry material	4.304	3.906	4.068	3.745	3.163	2.110

The magnitude of the changes in the protein content of *Acer Negundo* is in harmony with that of *Coleus*, except that the changes in the latter are much more precipitous.

MEYER (38) made macroscopic tests for proteins in the leaves of *Tropaeolum* by means of the xanthoproteic reaction. The natural color of the leaves was noted and compared with the depth of color which was produced by the xanthoproteic test. He found that as the green color of the leaf disappeared, the xanthoproteic reaction became less and less, or, in other words, as the protein of the chloroplast decreased, the chlorophyll in the chloroplast decreased also.

TABLE XI

XANTHOPROTEIC REACTION OF NORMAL ILLUMINATED
GROWING LEAVES OF *Trapaeolum*

Dark green leaves....................................5 to 4*
Green..3 to 4
Bright green...3
Yellow green..2 to 3
Yellow..2
Bright yellow.......................................1 to 2

*The larger the number the greater the amount of protein present.

In the leaves of the plane tree (*Platanus occidentalis*) TUCKER and TOLLENS found that the protein nitrogen decreased gradually

per unit area from July 15 until November 5. Over three-fourths of the nitrogen disappeared in this time.

In analyzing the leaves for total nitrogen when nitrates are present the method as described in Bulletin 107 (8) was used, with zinc as the reducing agent. Samples (10 gm.) of fresh leaves were used, and the results were calculated to grams of nitrogen per 100 gm. of fresh leaves. This analysis gives the total of the three forms (31) of nitrogen which may be found in organic matter (such as leaves), namely, nitrogen in a state of organic combination, nitrogen in ammonia or its combinations, and nitrogen in a more highly oxidized state as salts of nitrous or nitric acid. Table XII shows that there is a marked reduction in the total nitrogen content of the leaf with mottling. Boncquet (5) found that the total

TABLE XII

PROTEIN NITROGEN+NITRATE NITROGEN+AMMONIUM SALTS

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of nitrogen per 100 gm. dry weight
Green..................	0.283	0.267	0.249	0.266	4.19
Mottled *A*..............	0.117	0.107	0.103	0.109	1.87
Mottled *B*..............	0.074	0.071	0.060	0.068	1.09

nitrogen calculated on the basis of ash was always less in the diseased leaves than in the healthy ones.

The amount of nitrogen present in the leaves as NO_3 was determined by the Schlösing-Wagner method as given in Bulletin 107. For each determination 25 gm. of leaves was used. The leaves were finely ground in a mortar with quartz sand, boiled for 2 hours, made up to a definite volume, filtered through cheesecloth, and the amount of nitrate estimated in an aliquot part. The amount of gas as nitric oxide set free was then measured in the burette of a Van Slyke apparatus (35), and the nitric oxide was absorbed in a Hempel pipette containing NaOH and $KMnO_4$. The residual gas was then measured in the burette. The difference gave the amount of nitric oxide, which was reduced to standard temperature and pressure, and was calculated to grams of nitrogen per 100 gm. of wet weight, as given in table XIII, which shows an

enormous reduction in the percentage of nitrate nitrogen with mottling.

TABLE XIII
NITRATE NITROGEN

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of nitrogen per 100 gm. dry weight
Green..................	0.004,28	0.002,78	0.002,68	0.003,25	0.0485
Mottled *A*..............	0.001,90	0.000,80	0.000,72	0.001,14	0.0196
Mottled *B*..............	0.000,05	0.000,10	0.000,05	0.000,07	0.0011

Table XIV summarizes the results given in tables IX, XII, and XIII, and shows the following changes in the nitrogen compounds of the leaf with mottling. When the green leaves of *Coleus* were

TABLE XIV
COMPARISON OF NITROGEN FOUND

Form of nitrogen	Percentage wet weight	Percentage distribution of the three forms of N	Percentage dry weight
		Green	
Protein nitrogen.................	0.213	80	3.35
N as ammonium salts.............	0.050	18.8	0.79
N as nitrate....................	0.003	1.2	0.05
Total.......................	0.266	100.01	4.19
		Mottled *A*	
Protein nitrogen.................	0.081	74.3	1.39
N as ammonium salts.............	0.027	24.8	0.46
N as nitrate....................	0.001	0.9	0.02
Total.......................	0.109	100.01	1.87
		Mottled *B*	
Protein nitrogen.................	0.072	....	1.14
N as ammonium salts.............	−0.004	....	−0.05
N as nitrate....................	0.0001	....	0.0011
Total.......................	0.068	....	1.09

compared with the mottled, it was found that the amount of protein nitrogen, nitrogen as ammonium salts, and nitrogen as nitrates disappeared as the leaves mottled. The greatest decrease was found in the protein nitrogen, which showed that the protein

compounds were rapidly being broken down. In this connection SAMPSON (43) showed that the amino acid nitrogen of *Coleus* (based on dry weight) increased from 0.056 to 0.072 per cent as the leaves mottled. Table XIV shows further that when the leaves were completely mottled the nitrate nitrogen almost disappeared, which is in complete accord with the microchemical determinations. The table shows also that inorganic forms of nitrates were used up before the protein nitrogen was exhausted. PALLADIN shows that such was the case in starving plants. BONCQUET (4) believed that the plants he worked on were starving, due to lack of nitrogen.

Free ammonia was determined by a modification of the FOLIN method (35). Twenty-five gm. of the fresh leaves was finely ground with quartz sand, placed in an aeration tube, and ammonia-

TABLE XV

FREE AMMONIA

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Nitrogen per 100 gm. dry weight
Green..................	None	None	None	None	None
Mottled *A*..............	None	None	None	None	None
Mottled *B*..............	0.000,18	0.000,14	0.000,21	0.000,18	0.003,25

free air drawn through the tube for 2 hours. The ammonia which came from the leaves was absorbed in an aeration tube which contained 0.1N H_2SO_4. The ammonia set free was distilled off, after adding NaOH, and Nesslerized (33). Especial care was taken to use materials absolutely free from ammonia. The ammonia is calculated as grams of nitrogen per 100 gm. of wet weight. For the analysis leaves were selected which were free from mechanical injuries or drying at the tips. The mottled leaves *A* still had some green in them, while those of *B* were wholly yellow.

Other forms of ammonia were determined, such as albuminoid ammonia. The method as outlined by MASON (33) was followed, in which 10 gm. of the fresh leaves was distilled in the presence of NaOH and $KMnO_4$, 600 cc. of distillate distilled off and then a portion of it Nesslerized. The results calculated as grams of

nitrogen per 100 gm. of wet weight show that the mottled leaves had much less of the albuminoid ammonia.

Distillation of the leaves with Na_2CO_3 was also undertaken. Twenty-five gm. of the leaves was distilled in a flask with 2 gm.

TABLE XVI

ALBUMINOID AMMONIA

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of nitrogen per 100 gm. dry weight
Green.................	0.050	0.120	0.064	0.079	1.18
Mottled A.............	0.019	0.035	0.019	0.024	0.41
Mottled B.............	0.018	0.024	0.013	0.018	0.29

of Na_2CO_3 until 600 cc. of distillate was collected. An aliquot portion of it was Nesslerized and calculated as grams of nitrogen per 100 gm. of wet weight. Table XVII shows that the mottled leaves were lower in the amount of ammonia set free.

TABLE XVII

AMMONIA FROM DISTILLATION WITH Na_2CO_3

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of nitrogen per 100 gm. dry weight
Green.................	0.012	0.0044	0.019	0.012	0.18
Mottled A.............	0.0073	0.0031	0.0095	0.0066	0.11
Mottled B.............	0.0056	0.0040	0.0064	0.0053	0.085

Nitrites were determined by the method as given by DAVISSON (11). Attempts were made to determine the amount of nitrite by colorimetric methods (29), but the presence of anthocyanins which could not be precipitated out with lead subacetate interfered.

TABLE XVIII

NITROGEN AS NITRITE

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of nitrogen per 100 gm. dry weight
Green.................	None	None	None	None	None
Mottled.................	0.001,12	0.001,32	0.001,42	0.001,29	0.021,5

The fresh leaves (25 gm.) were ground finely with quartz sand, placed in the flask, and titrated as suggested by DAVISSON.

ASO and SEKINE (3) found that there was about 0.000,78 per cent N in the healthy buds of *Sagittaria sagittifolia*, when calculated on the basis of wet weight, or 7.8 parts N per million of material. In mottled *Coleus* leaves there are 12.9 parts per million, but not a detectable amount in green leaves. BONCQUET (4) reports the presence of nitrite and ammonia in various plants which were diseased. The diseases were of the physiological type such as curly leaf of sugar beets, curly dwarf of potatoes, mottled leaf of potatoes, and mosaic disease of the tobacco. He believes that

TABLE XIX

CARBOHYDRATES

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams per 100 gm. dry weight
	A. M.				
Green...................	0.890	0.855	1.050	0.932	13.93
Mottled *A*..............	0.740	0.820	0.685	0.748	12.89
Mottled *B*..............	1.110	1.090	0.935	1.044	16.84
	P. M.				
Green...................	1.435	1.370	1.430	1.412	21.08
Mottled *A*..............	0.780	0.850	0.825	0.822	14.17
Mottled *B*..............	1.020	1.040	1.155	1.073	17.30

nitrogen starvation is brought about by bacterial reduction of the nitrates to nitrites and ammonia, after the nitrates have been taken up by the roots. The response of the plant to the stimulus, he says, is manifested in biochemical, physiological, and morphological changes.

The amount of carbohydrate material in the leaves was estimated by first boiling (2.5 hours) 10 gm. of the freshly ground leaves in 100 cc. of water, to which 10 cc. of HCl (sp. gr. 1.125) was added. The sugars were then determined as dextrose by the BERTRAND (31) volumetric method. The calculations are given as grams of dextrose per 100 gm. of wet weight. Sugars were determined both in the morning and in the evening after a bright day,

to ascertain the relative amounts of photosynthetic activity in the green and mottled leaves.

The green leaves gained 0.48 gm. (as dextrose) during the day, mottled *A* gained 0.074 gm., and mottled *B* 0.029 per 100 gm. of wet weight, which shows that the photosynthetic activity was greatly reduced by mottling. In the morning the carbohydrate content of the mottled leaves in all probability consisted mainly of substances of an aplastic nature, such as the hemicellulose of the cell wall which by hydrolysis forms galactose, xylose, mannose, etc. Since the carbohydrate content probably was made up mainly of substances of an aplastic nature, one would not expect them to be exhausted by respiration or to be translocated from the leaf. WILLSTÄTTER (table XXVIII) has shown that the carbon dioxide assimilated per hour was greatly reduced as the leaves yellowed.

Water content and ash

For each determination 20 gm. of fresh leaves was used; the leaves were dried at 100° C. and then ashed at a dull red heat.

TABLE XX

DRY WEIGHT

Leaf	Sample no. I	Sample no. II	Average
Green...............	7.22	6.88	7.05
Mottled *A*............	5.80	5.58	5.69
Mottled *B*.............	5.79	6.60	6.20

This decrease in dry weight is in harmony with the work of BONC-QUET, or, in other words, leaves of mottled plants have a higher water content. WILLSTÄTTER (table XXVIII) showed that the water content of the leaves increased as yellowing progressed. This is due either to the fact that the materials were transported from the leaf or that respiration decreased the amount of dry matter present.

Table XXI shows that the percentage of ash when calculated on the dry weight of the leaves increased 3–7 per cent during mottling. Analyses of leaves as given by SWART (48) show that as a rule the yellow leaves gave a larger percentage of ash than

the green ones.   One might construe this to mean that materials are not transported from the leaf as it yellows, for if they were transported, presumably the salts would be carried along and the ash would decrease.   It seems more probable that since the assimilative activity is reduced, the respiratory products pass off as gas and so leave the salts behind.   BONCQUET (4) found that plants affected with nitrogen starvation have a higher percentage of ash (when based on dry weight) than normal plants, as is shown by beets, tobacco, and potatoes.

SCHULZE and SCHÜTZ (44) have shown that the ash content of leaves of *Acer Negundo* increases as autumn approaches.   The

TABLE XXI

Ash

Leaf	Sample no. I	Sample no. II	Average	Grams ash per 100 gm. dry weight
Green.....................	1.05	0.89	0.97	13.8
Mottled *A*................	0.97	0.93	0.95	16.7
Mottled *B*................	1.23	1.32	1.28	20.7

TABLE XXII

Ash in dry substance

	May 7	June 6	July 5	August 2	September 3	September 25
Percentage of ash.........	7.23	7.52	8.06	9.17	9.43	11.29

ash of the leaves increased 4 per cent, while the ash of *Coleus* leaves increased 3–7 per cent.   Ash of the plane tree leaves per unit area of leaf was shown by TUCKER and TOLLENS (49) to increase gradually until October 8, after which there was a slight decrease.

Catalase

In determinations of catalase activity only the blades of *Coleus*, exclusive of the primary veins, were used.   For each determination 0.5 gm. of the leaf material was ground for 2 minutes with a little quartz sand and powdered calcium carbonate as a neutralizer. The determination was run according to the method described by

APPLEMAN (1), using 10 cc. of dioxogen as the hydrogen peroxide and suspending the plant material in 10 cc. of water. Tables XXIII and XXIV show the cubic centimeters of oxygen liberated per 0.5 gm. of fresh weight of leaves at 25° C. during 10 minutes of activity. Table XXIII shows that the catalase activity gradually

TABLE XXIII

EFFECT OF AGE OF LEAF ON QATALASE ACTIVITY

OXYGEN LIBERATED

82.0 cc............................Top pair of leaves
88.5..................................Second pair of leaves
93.0..................................Third pair of leaves
86.0..................................Fourth pair of leaves
80.0..................................Fifth pair of leaves
74.0..................................Sixth pair of leaves
51.5*..................................Seventh pair of leaves
32.0†..................................Eighth pair of leaves

* Leaves still green.
† Leaves half mottled.

increased and reached a maximum in the third or fourth pair of leaves, and then decreased gradually until the leaf mottled. With mottling of the leaf it was noticed that the catalase activity dropped enormously.

From table XXIV it is evident that as the leaves mottled the catalase activity decreased greatly, even reaching a value of less

TABLE XXIV

COMPARISON OF CATALASE IN GREEN AND MOTTLED LEAVES

COMPLETELY MOTTLED	GREEN
6.3	103.0
10.0	95.0
8.0	90.0

than 1/10 that of the healthy leaves. The mottled leaves were taken from the plant 3 or 4 nodes below the green ones, and therefore age would be a factor, but it is not sufficient to account for the enormous decrease in the catalase activity.

An analysis of the leaves was made to determine the amount of nitrogen and phosphates in the F_1, F_2, and F_3 of the three types of leaves. Each morning the leaves were collected until 80 gm. was obtained for each sample; the leaves as collected were preserved in 95 per cent alcohol in ground glass stoppered, wide-mouthed bottles, and kept there until needed for analysis. An extraction

was made in 95 per cent alcohol at the temperature of the boiling solvent for 20 hours, and then for 4 hours in ether at the temperature of the boiling ether. The residue was dried, weighed, and called F_3, the ether-alcohol extract was F_1 and F_2 combined. The ether-alcohol extract was evaporated to dryness in a Freas vacuum oven at 70° C., weighed, and the portion of it which was soluble in anhydrous ether at room temperature was called F_1, and the weighed residue F_2. Aliquot parts of the three fractions were analyzed and results were calculated to grams of N or P per 100 gm. of fresh leaves.

TABLE XXV

PROPORTIONS OF VARIOUS FRACTIONS IN GREEN AND MOTTLED LEAVES, WEIGHTS OF F_1, F_2, AND F_3

Leaf	Sample no. I	Sample no. II	Sample no. III	Average
Green F_3	4.41	4.78	4.60	4.60
Green F_2	1.39	1.23	1.38	1.33
Green F_1	0.63	0.65	0.69	0.66
Total dry weight	6.43	6.66	6.67	6.59
Mottled A, F_3	3.66	3.94	3.74	3.78
Mottled A, F_2	1.61	1.46	1.68	1.58
Mottled A, F_1	0.16	0.13	0.10	0.13
Total dry weight	5.43	5.53	5.52	5.49
Mottled B, F_3	4.53	4.77	4.31	4.54
Mottled B, F_2	1.55	1.55	1.38	1.49
Mottled B, F_1	0.20	0.40	0.39	0.33
Total dry weight	6.28	6.72	6.08	6.36

In comparing the weights of F_3, it is seen that there was a decrease in weight as the leaves mottled, while the weight of F_2 increased and F_1 greatly decreased. The decrease in weight of F_3 is accounted for by the fact that the carbohydrate synthesis is considerably reduced, as was shown in table XIX, where the carbohydrates of the leaf were compared in the morning and in the evening. The increase of the weight of F_2 can be accounted for by the accumulation of salts in the older leaf. The results of F_1 showed that the sulphatides, phosphatides, nucleo-proteins, fats, etc., were greatly reduced in the mottled leaves, and many of these were nitrogen complexes.

The nitrogen in F_3 was reduced one-half or more, the nitrogen in F_2 was changed very little, while the nitrogen entirely disappeared in F_1 as the leaves mottled. This would mean that such compounds as nucleoproteins, glycoproteins, phosphoproteins, albumins, and globulins were rapidly being broken down, while the amount of derived proteins, amino acids, prolamines, ammonia compounds, and other nucleic acid metabolic products remained practically constant. Since the nitrogen in F_1 practically disappeared, it would seem that the phospholipins and amines were breaking down,

TABLE XXVI

NITROGEN OF VARIOUS FRACTIONS IN GREEN AND MOTTLED LEAVES

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of nitrogen per 100 gm. dry weight
Green F_3...............	0.17	0.17	0.19	0.18	3.91
Green F_2...............	0.018	0.017	0.021	0.019	1.43
Green F_1...............	0.005	0.002	0.004	0.004	0.61
Total dry weight......	0.193	0.189	0.215	0.203	....
Mottled A, F_3..........	0.06	0.06	0.08	0.07	1.86
Mottled A, F_2..........	0.017	0.018	0.027	0.021	1.33
Mottled A, F_1..........	None	None	None	None	None
Total dry weight......	0.077	0.078	0.107	0.091	....
Mottled B, F_3..........	0.05	0.05	0.05	0.05	1.10
Mottled B, F_2..........	0.015	0.010	0.006	0.010	0.67
Mottled B, F_1..........	None	None	None	None	None
Total dry weight......	0.065	0.060	0.056	0.060	....

and that the plant must be drawing upon its last sources of nitrogen before death ensues. In this connection the work of KORAPETOVA and SOBASHNIKOVA (40) is very significant. They grew seedlings of rye and barley in inadequate nutrient solutions and found that the total amount of proteins decreased as growth progressed. In mottling of *Coleus* one likewise seems to be dealing with inadequate (especially nitrogen) nutrition. Here too proteins are decomposed, probably owing to the shortage of nitrogen.

In mottled leaves the phosphates of F_3 were reduced to one-third that of the green leaves, while the phosphates in F_2 increased

about 30 per cent, and those in F_1 had almost disappeared. As the main part of the phosphorus in F_3 probably existed as phosphoprotein, there was apparently a hydrolysis of this going on to form hydrolytic products of nucleic acids. Since little or no phosphorus was found in F_1, apparently the phosphatides had nearly all disappeared. Germination (40) in darkness appears to be correlated with a pronounced decomposition of phosphorus containing proteins. Apparently during the mottling of *Coleus* there is a similar phenomenon. PALLADIN (40) showed that as

TABLE XXVII

PHOSPHATES

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of phosphorus per 100 gm. dry weight
Green F_3................	0.0330	0.0184	0.0213	0.0242	0.526
Green F_2................	0.0013	0.0008	0.0007	0.0009	0.068
Green F_1................	0.0027	0.0026	0.0030	0.0028	0.425
Total dry weight......	0.0370	0.0218	0.0250	0.0279	....
Mottled A, F_3..........	0.0069	0.0068	0.0056	0.0064	0.169
Mottled A, F_2..........	0.0018	0.0014	0.0018	0.0017	0.107
Mottled A, F_1..........	None	None	None	None	None
Total dry weight......	0.0087	0.0082	0.0074	0.0081	....
Mottled B, F_3..........	0.0067	0.0095	0.0080	0.0081	0.178
Mottled B, F_2..........	0.0018	0.0012	0.0012	0.0014	0.094
Mottled B, F_1..........	None	0.0010	0.0010	0.0007	0.212
Total dry weight......	0.0085	0.0017	0.0102	0.0102	....

proteins decomposed the inorganic phosphates increased. From table XXVII it is apparent that the phosphoproteins were being rapidly decomposed as the leaves mottled. If the phospholipins are estimated according to CZAPEK (10), by multiplying the amount of magnesium pyrophosphate in the ether extract by 7.27, then 0.073 per cent of the wet green leaves and 1.009 per cent of the dry weight is lecithin. In the mottled leaves the phospholipins have almost entirely disappeared. The ratio of phosphorus (table XXVII) to nitrogen (table XXVI) is about 1 to 7 in the green leaves, while in the mottled leaves the ratio is about 1 to 11.

Leaf pigments

CHLOROPHYLL *a* AND *b*.—The amount of leaf pigments of *Coleus* was compared with the amount of leaf pigments in the lilac, according to the method given by WILLSTÄTTER and STOLL (53). The ether extracts of the pigments were compared spectroscopically, and the amount of chlorophyll present in the lilac and in the *Coleus* was compared by the width of the absorption bands in the red end of the spectrum. In this comparison the band lying between the Fraunhofer lines *B* and *C* was used, since it was the most distinct. The extract of the *Coleus* leaves absorbed the rays from 685.5 $\mu\mu$ to 674.5 $\mu\mu$. The chlorophyll solution from the lilac leaves was then diluted until it gave an absorption band of the same width. It was found that the chlorophyll *a* and *b* content of the healthy green leaves of lilac was five times as great as the chlorophyll content of the healthy green leaves of *Coleus*. Not even a trace of absorption in the red end of the spectrum was observed in the ether extract of the completely mottled leaves.

The four leaf pigments (chlorophyll *a* and *b*, carotin, and xanthophyll) of green and completely mottled leaves of *Coleus* and of green leaves of lilac were then separated according to the method given by WILLSTÄTTER and STOLL,[1] and the extractions tested spectroscopically to make sure of the purity of each extraction. The amount of each of the four pigments in the various leaves was then compared by means of a Schreiner colorimeter. The amount of chlorophyll *a* and *b* in the lilac leaves was taken as a standard, and the amount of green pigments in the *Coleus* leaves was compared with that of the lilac leaves. The writer realizes the limits of such a standard and regrets that there is not some stable dye or color which would serve as a basis for determining the exact amount of chlorophyll pigments in a leaf. The extraction of the pure pigments is a rather lengthy and expensive process, and when the pure pigments are once obtained some of them apparently do not keep well.[2] By colorimetric comparison the lilac leaves were

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[1] A method for the quantitative estimation of the four pigments of green leaves will be published later.

[2] Preparation of these pigments, their keeping qualities, and some spectrophotometric data will be published soon.

found to have about five times as much phytochlorine as the *Coleus* leaves (based on the wet weight). The same relation was found to hold for phytorhodin *g*. This means that the two leaves bear *a* and *b* chlorophyll in the same proportions, but that the lilac has about five times as much of each as the green *Coleus*. Assuming that the lilac leaves contain 0.8 per cent (the average percentage for green leaves) of chlorophyll, then *Coleus* leaves contain only 0.16 per cent of chlorophyll, based on the dry weight of the leaves.

Table XXVIII, quoted from WILLSTÄTTER, shows the relation between autumnal yellowing, chlorophyll content, and photosynthetic activity. These changes agree generally with the writer's results in the mottling of *Coleus* in mid-season.

TABLE XXVIII

CHANGES AS LEAVES YELLOW IN AUTUMN (LEAVES ALL OF SAME SPECIES)

Leaf	Date	Dry weight in gm.	Chlorophyll content	CO_2 assimilated per hour
Deep green	July 30	1.55	19.7	0.080
Green	September 17	1.55	12.5	. . . .
Green with yellow spots	October 5	1.45	7.8	0.064
Almost yellow	October 19	1.35	2.1	0.010

In alfalfa hay JACOBSON (23) has shown that 0.68 per cent of chlorophyll and 0.28 per cent of yellow coloring matter were present. The chlorophyll was estimated after the method of MARCHLEWSKI, and was shown to contain 66 per cent neochlorophyll and 34 per cent allochlorophyll. He believed that this ratio would vary, depending upon the conditions of growth.

XANTHOPHYLL.—The xanthophyll in the leaves was estimated by comparing the extracted pigment with a standard solution of potassium dichromate, as recommended by JÖRGENSEN and STILES (26). There was found to be present in 1000 gm. of fresh lilac leaves 0.273 gm. of xanthophyll, in the green *Coleus* leaves 0.087 gm., and in the mottled *Coleus* leaves 0.239 gm. From this it is seen that green *Coleus* has about one-third as much xanthophyll as lilac, while fully mottled *Coleus* has nearly as much as lilac. The amount of xanthophyll present in lilac was about the same as that present in *Sambucus nigra* (0.250). The amount of

xanthophyll in green *Coleus* leaves was about one-third of the amount present in most leaves.

CAROTIN.—Mottled *Coleus* leaves were found to contain about 0.3575 gm. of carotin per 1000 gm. of fresh leaves, while green *Coleus* leaves had 0.0894 gm. and green lilac leaves had 0.1324 gm. None of the leaves analyzed by WILLSTÄTTER gave as much carotin as did the mottled *Coleus* leaves. Poplar leaves (0.097) had about the same amount of carotin in them as did green *Coleus* leaves, while the leaves of *Sambucus nigra* (0.134) and *Fagus silvatica* (0.131) had about the same amount of carotin in them as did lilac leaves.

The ratio of carotin in lilac leaves was found to be $\dfrac{0.1324}{0.2730}$, or 0.48; for green *Coleus* leaves $\dfrac{0.0894}{0.0870}$, or 1.05; for mottled *Coleus* leaves $\dfrac{0.3575}{0.2390}$, or 1.49. WILLSTÄTTER found that the average ratio was 0.603 ± 0.1. Even in green *Coleus* leaves the carotin was higher than the average, and the ratio in the mottled leaves was greatly increased over what it should be if the leaf were normal as to its yellow pigments.[3]

Discussion of leaf pigments

Various theories have been proposed to explain how the pigments change as the leaf yellows. SWART (48) found that yellowing of leaves which are dying begins in that part of the parenchyma which is farthest from the vascular bundles, and takes place last in the largest vascular bundles. In connection with the disappearance of chlorophyll on aging of the leaf SWART mentioned three possibilities: the chlorophyll either was transported or it remained in the leaf when it broke down, and if it broke down in the leaf the decomposition products either were transported to the stem or else remained in the leaf. He thought that chlorophyll in the form of decomposition products passed from the leaves into the stem, while the yellow pigments remained in the leaf.

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[3] For comparative work on the yellow pigments Lovibond slides have been found to be quite satisfactory.

STAHL also believes that the products of chlorophyll decomposition do not remain in the leaf, but diffuse through the veins to the stem. He gave as proof the fact that if the veins of a *Ginkgo* leaf are severed, the chlorophyll remains longer than it does in a control leaf. Similar experiments were conducted on the leaves of *Coleus* by the writer, but the leaves yellowed just the same.[4] The types of leaves are entirely unlike, hence it is not surprising that the results were different. STAHL believes further that, since the yellow pigments (carotin and xanthophyll) consist of carbohydrate materials only, they were not needed by the plant and so were left behind, but the green chlorophyll pigments which contained magnesium and nitrogen were decomposed, and these elements were carried away to meet the requirements of the plant.

SWART has shown that it is unlikely that magnesium is withdrawn from the leaf. Table V shows that the plant does not lack magnesium, and consequently there would be no occasion for it to draw upon its chlorophyll supply for the very small quantity which is present in the chlorophyll molecule. On the other hand, all the data tend to show that nitrogen is the element lacking, and therefore it is quite possible that either the chlorophyll would be prevented from forming, or if formed would be decomposed, if the law of mass action plays any part at all in the process.

The situation is summarized by MEYER (38) in the case of yellowing of *Tropaeolum* leaves. As the leaves age they become weakened. This weakening of the leaves results in curtailed assimilation, which is limited because the chloroplasts become smaller and the organs are weakened. This weakening of the organs is a primary cause, while the decomposition of the protein follows because of this. Lastly, the decomposition of the protein accelerates the yellowing of the leaf. MEYER believes that the chlorophyll decomposes and is then borne away, while the yellow pigments remain as they were in the leaf, neither increasing nor decreasing in quantity. Since he made no quantitative determinations on the pigments of the leaves, it is easy to see how he might have deduced such a conclusion in regard to the yellow

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[4] On the leaves of *Ginkgo*, during the summer and autumn of 1920, at Washington, D.C., the experiments of STAHL could not be confirmed.

pigments. Had MEYER availed himself of methods of estimating the yellow pigments, he doubtless would have reported differently.

KOHL (30) has written extensively on carotin. He has shown that in *Vicia Faba* seedlings carotin forms and increases in the dark; in the light, at a low temperature, chlorophyll formation is suppressed, while the carotin increases; increase of light and temperature accelerates chlorophyll formation. Working with several species of plants, he concluded that etiolated plant organs owe their color almost exclusively to carotin. When some etiolated plants greened, the carotin content was found to increase sometimes as much as 125 per cent, and in all cases was found to increase to some extent. He did not believe that chlorophyll was formed at the expense of the carotin, however, nor did he think the chlorophyll was changed to carotin in autumnal coloration. His experiments on coloration led him to conclude that the carotin content (evidently including carotin and xanthophyll) of the leaves increases. His results show that the carotin content of old leaves of *Sambucus nigra* is to the carotin content of young leaves of the same plant as 183:170. Color changes which he describes are very similar to those described by MEYER in autumnal yellowing.

STOKLASA, SEBOR, and SENFT (47) believe that the autumnal changes of color depend on the hydrolytic fission of chlorophyll and the formation of phaeophytin and phosphatides; these substances, which themselves have a brownish color, allow the red and yellow of carotin and of xanthophyll to appear. The colorless lecithin and choline derivatives are not combined with chlorophyll, but are merely admixed and possibly intimately associated with the chlorolecithins. The writer has shown that the yellow pigments in *Coleus* greatly increase as the leaf mottles. The yellow pigments are not simply left behind when the other pigments are translocated, but are being continually formed.

WILLSTÄTTER stated that the proportion of chlorophyll *a* and *b* to the yellow pigments was 3.07 to 1 in sun leaves, while in shade leaves it was 4.68–6 to 1. IWANOWSKI (22) found that less chlorophyll was broken up by light when the yellow pigments were in greater concentration. He concluded that the protective action of the yellow pigments could no longer be doubted. The yellow

pigments absorb blue and especially violet rays, whose power to break down chlorophyll is especially high. It is of interest to notice how the carotin content varies under different conditions. The amount of carotin in a leaf varies according to the seasons of the year (40), being greatest during the flowering period in nettles and horse chestnuts. In vetch (40) about five times as much carotin exists in the green leaves as in the etiolated ones.

EWART (16) has shown that when mustard seedlings were grown in the absence of carbon dioxide, more carotin was produced than when carbon dioxide was present. He remarks that his method of analysis was not wholly accurate, however, and that during the separation of the pigments the losses were so great that the exact estimation of the amounts originally present was impossible. The seedlings grown in air deprived of carbon dioxide were

TABLE XXIX

AMOUNTS OF PIGMENTS PRODUCED IN MUSTARD SEEDLINGS
(PER 100 GM. OF FRESH MATERIAL)

CO_2	Chlorophyll	Carotin	Xanthophyll
Present.....................................	0.474	0.11	0.15
Absent......................................	0.271	0.275	0.12

smaller, darker, and more bluish green. This blue green seemed to be due not to an excess of chlorophyll, but rather to the more compact character of the tissues, and it appears that chlorophyll develops most rapidly when its normal functional activity can be exercised. EWART believes that he is justified in concluding that the carotin supplied at least a part of the carbon and hydrogen for the construction of chlorophyll.

KOHL (30) showed that etiolated seedlings contained carotin in abundance, and even doubted whether any other pigment was present. He found that the percentage of carotin did not decrease when the etiolated seedlings were exposed to light and chlorophyll was formed. He thus denied that carotin was converted into chlorophyll as was believed by EWART. EWART states that while food materials were abundant the production of carotin continued at a greater rate than it was used in the formation of chlorophyll.

He showed that etiolated wheat seedlings contained 8-10 parts of carotin to one of xanthophyll, and that leaves of *Hordeum murinum* when kept in darkness turned yellow or yellowish red. When analyzed for plant pigments, the leaves were found to contain chlorophyll, xanthophyll, and carotin in the proportions 1, 3, and 12, respectively, and in addition a red pigment, which was apparently a flavone, believed to be a decomposition product of chlorophyll. His suggested explanation of the greening of etiolated plants is unique. When an etiolated plant turns green in light, the carotin undergoes photo-oxidation. The bleached carotin residue combines with glaucophyllin, converting it into the tri-carboxylic chlorophyll.

WILLSTÄTTER found that a weak alcoholic oxalic acid solution splits (in the cold) magnesium out of the chlorophyll molecule. On this basis SWART assumes that the decomposition of chlorophyll in yellowing leaves is due to acids, thus splitting the chlorophyll molecule. This postulates an increase of acidity with yellowing, a theory for which there is no evidence. SAMPSON (43), in testing the acidity of *Coleus* leaves, found that fresh yellow leaves in the act of abscissing had an acidity equivalent to 0.0069 cc. of N acid per gram of wet weight, while fresh green leaves had an acidity equivalent to 0.0089 cc. of N acid. Since the green leaves are more acid (at least as measured by their base absorbing power) than the yellow ones, if SWART'S assumption is correct, one would expect to see the top leaves of the plant yellow instead of green. It is hardly probable that the splitting of magnesium out of the chlorophyll molecule, due to acid accumulation, is the first step in chlorophyll decomposition. PALLADIN (40) pointed out that carbohydrates are essential to the formation of chlorophyll. From the sand culture experiments with *Coleus* one could hardly say that the deficiency in carbohydrates caused the chlorophyll to disappear. It would be more accurate to say that the carbohydrate output was decreased, owing to the deficiency in chlorophyll.

WIESNER (52) supposed that the chlorophyll in the living leaf was dissolved in an oil, in which the concentration of chlorophyll was very high and the decomposition very low. IWANOWSKI (21) agreed with WIESNER in regard to the concentration of the chloro-

phyll, and has proved that plants with much chlorophyll show little or no breaking up of chlorophyll by light, and that plants with little chlorophyll (as *Elodea*) show as much as 31 per cent of the chlorophyll broken up by light in seven hours. IWANOWSKI also showed that colloidal solutions were about sixteen times as light stable as molecular solutions, and the more concentrated the colloidal solution the more light stable it becomes. HERLITZKA (20) also found that the chlorophyll exists in the leaf in the colloidal state.

In view of these facts one might expect light lability to enter as a factor in the decomposition of chlorophyll in *Coleus*, for the chlorophyll is only one-fifth as concentrated as it is in most other sun plants.

BORESCH (6) in his experiments on algae (*Phormidium corium*) showed that the algae when grown on nutrient media changed from a dark green, after two months, to gold or red brown. Addition of more of the nutrient media caused them to resume again their green color. He believes that the change of color back to the natural green was due to the presence of the nitrogen in the potassium nitrate. Any nitrate, ammonium salt, or other nitrogen compound would do the same. Other plants, such as *Chlamydomonas*, *Hydrodictyon*, and *Oedogonium*, depend upon nitrogen compounds for their existence, and also the building and accumulation of chlorophyll depends upon the available nitrogen supply. Extractions of the pigments were made by BORESCH, who found that the green Cyanophyceae showed the normal colors (chlorophyll, phycocyan, and carotin), while the brownish extracts of these algae showed little chlorophyll and much of the carotin. He believes that the brown color was due to the breaking down of the chlorophyll and phycocyan, which are closely related in their origin. He also noted that the carotin increased as the chlorophyll and phycocyan broke down. In the case of higher plants, BORESCH noticed that nitrogen had a greening effect upon the plants. When nitrogen was failing and the leaves were getting yellow, additions of manure kept them green. ARTARI (2) found that chlorophyll formation and the quantity of chlorophyll depend upon the substratum. *Stichococcus bacillaris*, when grown in the dark with

nitrogen sources such as asparagin, peptone, and ammonium nitrate, greened, and when potassium nitrate was used it became pale or colorless. If *Stichococcus* were grown in the light and fed upon rich organic nutrients (maltose, glucose), it lost its chlorophyll. If the colorless algae which had been growing in the dark were placed in the light and given potassium nitrate as nutrient, they regained their normal color and the chromatophores became normal again. He did not know whether the chromatophores were built up anew or not. ARTARI also found that when he placed the colorless algal cells on a nutrient solution which contained either asparagin or ammonium nitrate, and placed them in the dark, the algae greened again.

TABLE XXX

COMPARISON OF GREEN AND YELLOW PIGMENTS IN LEAVES
OF HEMP PLANTS UNDER DIFFERENT TREATMENTS

Process	Chlorophyll	Carotin
Intense manuring..............	100	100
Complete manuring............	74	90
Nutrients lacking nitrogen......	38	57
Nutrients lacking phosphates...	71	80
Nutrients lacking potassium....	66	72
Nutrients lacking calcium......	72	90
No nutrients added...........	53	71

The effect of the various nutrient elements is perhaps best described by VILLE (51). He experimented upon the effect produced on the color of many field plants by a deficiency in nitrogen, calcium, phosphate or potassium, and no manuring. He found that nitrogen affects chiefly the color of the plants, and if it is deficient the plants become brown, while if the dose of nitrogen is increased or diminished, the color increases or diminishes accordingly. He made crude extracts of the leaves of hemp and compared the green and yellow pigments in them. Table XXX shows the results of VILLE's experiments.

The *Coleus* here studied seems to be on the verge of nitrogen starvation at all times under ordinary greenhouse conditions. It is only with considerable nitrate additions that the nitrogen supply of the leaf can be maintained sufficiently to avoid chlorophyll

decomposition. It is a great nitrogen user, and it is difficult to know what use is made of all the nitrates it consumes. One wonders whether there is a denitrifying process going on within the leaves which keeps the nitrates more or less depleted, and in case of lack of continuous additions completely removes them. This or some draft on the nitrogen of the leaf leads to a decomposition of nitrogen compounds of the leaf, including proteins, phospholipines, and chlorophyll, the decomposition of the latter producing the mottling. All the experimental evidence points to the shortage of nitrogen as the cause of chlorophyll decomposition in the plant studied, a plant very prone to mottling. One would hardly expect the same limiting factor to determine chlorophyll decomposition in plant organs in all cases, but many of the cases of chlorophyll loss discussed, whether involving loss from plants grown in cultural solutions, or in soils in the midst of the growing season, or loss accompanying autumnal coloration, point in the same direction. In the *Coleus* studied it is evident that there is little ground for SWART's view that high acidity of the leaves leads to a decomposition of chlorophyll by splitting magnesium out of the molecule. Cultures with cuttings of this variety of *Coleus* also show that under ordinary cultural conditions the plant bears little margin of phosphate supply, while it bears a great excess of calcium, magnesium, and iron. The narrow margin of phosphate supply does not manifest itself in mottling, but only in limited growth. The narrow margin of nitrogen, on the other hand, manifests itself both in limited growth and in mottling. If IWANOWSKI is correct in his assertion that carotin and xanthophyll render chlorophyll more nearly light stable, an increase in these pigments during mottling may act in a protective way against decomposition of chlorophyll.

From the various investigations presented two things are of striking interest: the decomposition of chlorophyll and the great amounts of yellow pigments which are present when chlorophyll is absent, whether it is in etiolated plants, algae grown in the dark, plants which have poor nutrition, or when leaves mottle. Since the carotin is present in greater amounts when chlorophyll is absent, and since carotin apparently decreases as chlorophyll

increases, one would naturally seek the cause of this, and is led to investigate the relationships which may exist between the various plant pigments.

EWART (16, 17) believes that carotin after photo-oxidation or partial disintegration forms a massive hydrocarbon combination (the phytol radicle of chlorophyll) whose addition is necessary to convert the dicarboxylic glaucophyllin into the tricarboxylic chlorophyll. Perhaps this might explain the disappearance of carotin as chlorophyll forms. The bridge between the two yellow pigments is not so difficult to cross, for one of them (xanthophyll) is simply the oxidation product of the other (carotin).

Some believe that chlorophyll may act as a sensitizer, and others that it may act as a photic or lytase enzyme which converts carbon dioxide and water into carbohydrates. EWART (16) has shown that chlorophyll may act as an enzyme according to the following three equations:

(1) $2 C_{31} H_{29}N_4Mg(COOH)(COOCH_3)(COOC_{20}H_{39}) + 36CO_2 + 16H_2O = 2 C_{40}H_{56}O_2 + 44O_2 + 2C_{31}H_{30}N_4Mg(COOH)_2$ Amorphous chlorophyll+carbon dioxide and water would form xanthophyll or carotin, oxygen, and glaucophyllin. (2) $C_{40}H_{56}O_2 + 24H_2O + 7O_2 = 2 C_{20}H_{39}OH + 3C_6H_{12}O_6 + 3C_6H_{12}O_6 + 4 HCHO$. Carotin or xanthophyll+water+oxygen equals phytol+levulose+glucose+ formaldehyde. (3) $2C_{20}H_{39}OH + 2C_{31}H_{30}N_4Mg(COOH)_2 + 4CO_2 = 2C_{31}H_{29}N_4Mg(COOH)(COOCH_3)(COOC_{20}H_{39}) + 3O_2$. Phytol+ glaucophyllin and carbon dioxide form amorphous chlorophyll and oxygen.

Reaction 2 takes place in the light with the aid of an oxidase enzyme.

Since many chemical reactions are reversible, and since phytol splits off the chlorophyll molecule easily, it is possible that the sugars and phytol may react to form carotin or xanthophyll, which would account for the greater accumulation of yellow pigments in autumn leaves and also for the disappearance of the chlorophyll. Film experiments carried out by EWART (17) showed that carbon dioxide combines with chlorophyll to form xanthophyll and a colorless waxy substance. The combination takes place in the presence of water and is accelerated by sunlight. It is thus seen

how the yellow pigments in the leaf may increase at the expense of the chlorophyll.

Bacteria

The leaves of *Coleus* were examined closely for the presence of bacteria. In the healthy green leaves a few bacteria of the coccus type were observed, while in the fully mottled leaves many bacteria of this type were found. A few of the bacillus type were also present in the cells. The presence of ammonia and nitrite can possibly be accounted for by their activity. Plates were made of the leaves under sterile conditions so as to get only those bacteria which were inside the cells, and always a much greater bacterial count was obtained from the mottled leaves. It is realized that the bacterial side of this question is really a problem in itself, and that this phase of the subject ought to be further investigated.

The juice of the mottled leaves was placed on the under side of the healthy green leaves and rubbed around; in other cases, in addition to rubbing the juice on the leaf, the veins were injured mechanically. The leaves in these cases mottled no sooner than did the leaves of the untreated plants, and they mottled in exactly the same manner as untreated plants. The writer believes that the bacteria get a better hold as the leaf weakens from nitrogen starvation. Evidently the organism present is a denitrifying one, which develops somewhat in the green leaf, and as the leaf weakens or mottles the organism develops more rapidly.

It will be worth while to summarize what other workers have discovered about certain bacterial and physiological diseases which in some respects appear to be similar to the mottling of *Coleus*. Freiberg (18) inoculated varieties of pumpkins, squash, watermelon, cucumber, citron, muskmelon, and others, and not a single infection resulted from his inoculations, yet during the same season other plants of these same varieties contracted the mosaic disease. Jagger (24) and Doolittle (13) report that the mosaic leaf disease of cucumber is transmissible by rubbing the healthy plants with crushed diseased leaves, and have proved that *Aphis Gossypii* transmits the mottled leaf disease of the cucumber. Smith and Boncquet (45) state that *Eutettix tenella* is the only carrier

of the disease-producing agent of the curly leaf of sugar beet. STEWART and REDDICK (46) report that the mosaic disease of beans is transmitted through the seeds, and that healthy seedlings rubbed with crushed diseased leaves showed infection four weeks later. McCLINTOCK (36) noticed that several varieties of lima beans mottle, while the larger variety growing with these does not mottle. He thought that the bean mosaic disease was carried by the seed. EDSON and SCHREINER (15) state that ORTON observed a potato disease characterized by bronzing and later browning of the leaf. This disease appeared in New Jersey, but was absent in areas which were treated with potash or stable manure. They believed that the primary cause was insufficient potash or perhaps an excess of nitrates in the presence of a minimum potash supply.

BONCQUET (5) found that the mottled leaf of beets, tobacco leaves, mottled potato leaves, and many other mottled leaves were associated with bacteria. The normal green leaves of these plants gave no nitrite or ammonia tests, while usually the mottled leaves showed the presence of nitrites and free ammonia. The fact that a leaf is yellow, however, is no sign that nitrite or ammonia is present in it. BONCQUET thought that the mottling of the leaves was a pathological disturbance brought about by the partial and local nitrogen starvation of the tissues. Nitrogen starvation in the leaves around nitrate-reducing foci explained the mosaic nature of the leaf diseases in which an abundance of nitrite was detected. Potato plants growing in soil rich in nitrate may yellow and mottle, owing to the presence of nitrate-reducing bacteria in abundance.

Summary

1. The leaves of *Coleus Blumei* (var. Golden Bedder) are very prone to mottling or loss of chlorophyll. Mottling progresses from the lower leaves upward. Mottling of the leaves takes place first at the edge of the leaf and progresses slowly toward the veins and to the base of the leaf. Usually the pair of leaves immediately above these mottles next, and so on as the plant grows.

2. In mottling the chloroplasts lose their green color, become reduced in size, and carry on very little photosynthesis.

3. In the usual greenhouse cultural conditions, this plant has within it phosphorus and nitrogen little in excess of its immediate needs. This was shown by cuttings grown in phosphate-free nutrient mixtures; failure to grow was noticeable, although the plant maintained a healthy green color. When the plants were transferred to nitrate-free mixtures, they failed to grow and also lost their natural green color.

4. Under usual cultural conditions these plants seemed to have within their tissue magnesium, calcium, and iron greatly in excess of their immediate needs. Cuttings grown in nutrient mixtures lacking any one of these elements grew and maintained their normal green quite as well as in the complete nutrient mixtures.

5. A deficiency of magnesium or calcium apparently has nothing to do with mottling.

6. More iron was found in all parts of the mottled than in the green leaf.

7. A deficiency in phosphorus caused a larger percentage of the leaves to drop than did a deficiency in iron, magnesium, calcium, or nitrate.

8. A deficiency in phosphorus caused more of the leaves to drop, while a surplus of phosphorus did not prevent them from falling if nitrogen was deficient.

9. The effect of adding nitrogen to a plant or withholding it was shown in a very few days by the change in color of the leaves.

10. Addition of a nitrogen compound (sodium nitrate) to a plant potted in soil kept the leaves on and the plant green, while the addition of iron, magnesium, calcium, or phosphate made very little change in the appearance of the plant.

11. In order to maintain a healthy condition and a green color the plants seemed to require more nitrate than other plants of which we have a record.

12. The mottled leaves always had a lower percentage of nitrate nitrogen, protein nitrogen, ammonium salts, and albuminoid ammonia than did the green leaves; mottled leaves had nitrites and free ammonia present in them.

13. The general appearance of the mottling was the same as that of the leaf of citrus fruit trees.

14. Mottling of the leaves greatly lessened the carbohydrate output.

15. The catalase activity of the leaves was very greatly reduced as the leaves mottled.

16. The dry weight of the mottled leaves was less and the ash greater than that of healthy green leaves.

17. In mottled leaves the weight of F_1 and of F_3 was less, while that of F_2 was more than that of the green leaves.

18. Protein nitrogen decreased in all of the three fractions as the leaves mottled, while phosphates decreased in F_3, increased in F_2, and almost disappeared in F_1.

19. The amount of chlorophyll (*a* and *b*) was about one-fifth of that of lilac leaves; the proportion of *a* to *b* was the same as in other plants (lilac).

20. The carotin and xanthophyll content greatly increased as the leaves mottled.

21. Bacteria were found within the cells of the mottled leaves, but it is not known whether they bear a causal relation to mottling or not.

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BUREAU OF PLANT INDUSTRY
    WASHINGTON, D.C.

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